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Effects of hydrogen sulfide on hypoxic pulmonary vascular structural remodeling

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Abstract

To study the role of hydrogen sulfide (H_2S) in hypoxic pulmonary vascular structural remodeling (HPVSR), a total of 24 Wistar rats were randomly divided into three groups: control group ($n=8$), hypoxia group ($n=8$) and hypoxia with sodium hydrosulfide (hy+NaHS) group ($n=8$). The mean pulmonary artery pressure (mPAP), plasma H_2S and the percentage of muscularized arteries (MA), partially muscularized arteries (PMA) and nonmuscularized arteries (NMA) in small pulmonary vessels were measured. Collagen I and III, elastin, transforming growth factor-β_3 (TGF-β_3), proliferative cell nuclear antigen (PCNA) and human urotensin II(U-II) expressions were detected by immunohistochemical assay. The mRNA expressions of procollagen I and III, matrix metalloproteinase-1 (MMP-1) and tissue inhibitor of metalloproteinase-1 (TIMP-1) were detected by in situ hybridization. The results showed that NaHS significantly increased plasma H_2S, decreased mPAP and the percentage of MA and PMA of small pulmonary vessels in rats under hypoxia. Meanwhile, NaHS inhibited the proliferation of pulmonary artery smooth muscle cells (PASMCs) represented by a decrease in the expressions of PCNA and human U-II in pulmonary artery wall. NaHS reduced the expression of collagen I and III, elastin and TGF-β_3 protein and decreased the expressions of procollagen I and III mRNA in pulmonary arteries of rats under hypoxia, but it did not impact the ratio of TIMP-1 mRNA to MMP-1mRNA in pulmonary arteries of rats under hypoxia. These data suggested that H_2S played an important role in the development of HPVSR.

Keywords: Hydrogen sulfide; Hypoxia; Pulmonary vascular structural remodeling; Proliferative cell nuclear antigen; Urotensin II; Collagen; Elastin; Matrix metalloproteinase-1; Tissue inhibitors of metalloproteinase-1

Introduction

Hypoxic pulmonary hypertension is an important pathophysiological process in the development of a variety of cardiopulmonary diseases (Vender, 1994). Hypoxic pulmonary vascular structural remodeling (HPVSR) is considered as the key pathologic basis of hypoxic pulmonary hypertension (Nakamura, 2001). The excess accumulation of extracellular matrix (ECM) in the wall of small pulmonary muscularized arteries as well as the increased proliferation of pulmonary vascular smooth muscle cells (PASMCs) is the main pathological process of HPVSR. Many scientists have paid great attention to the investigation of the pathogenesis of HPVSR. In the 1980's, nitric oxide (NO) and carbon monoxide (CO) were determined to be gaseous messenger molecules sharing common features such as low molecular weight, continuous release and quick dispersal and absorbance (Ignarro et al., 1987; McFau and McGrath, 1987). The above discoveries have been moving the research of HPVSR to a very new phase (Qi et al., 2001; Shi et al., 2003; Gong et al., 2004). Although the gaseous messenger molecules were found to be capable of relaxing vessels and interfering with vascular structural remodeling, the possible mechanisms responsible for the

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regulation of HPVSR during the development of hypoxic pulmonary hypertension still remain unclear. Hydrogen sulfide (H_2S) has been known as a toxic gas for a long time. However, it was recognized that H_2S could be endogenously generated from a reaction catalyzed by cystathionine-β-synthesis (CBS) and cystathionine-γ-lyase (CSE) (Stipanuk and Beck, 1982) and more interestingly, it was recently found to exert a variety of vascular biological effects such as inhibiting cultured vascular smooth muscle cell proliferation (Du et al., 2004) and acting as an opener of K_{ATP} channel (Geng et al., 2004; Zhao et al., 2001).

In our foregoing studies we observed that in a rat model of hypoxic pulmonary hypertension, the level of H_2S in plasma and its production rate in lung tissues and pulmonary arteries decreased markedly. The expression of CSE mRNA in lung tissue was also downregulated. After treatment with an H_2S donor, the level of H_2S in plasma and the production rate of H_2S in lung tissues and pulmonary arteries increased. At the same time, the elevated level of pulmonary artery pressure decreased and hypertrophy of the right ventricle and remodeling of the small pulmonary muscularized arteries were ameliorated. The above findings suggested that the down-regulation of the endogenous H_2S pathway might be involved in HPVSR (Zhang et al., 2003). However, the molecular mechanism by which H_2S regulates HPVSR is not fully understood. Based on the fact that the excess accumulation of extracellular matrix, such as collagen I and collagen III in the wall of small pulmonary arteries is the main component of hypoxic pulmonary vascular structural remodeling (Gong et al., 2004) and that H_2S was recently found to be able to regulate vascular smooth muscle cell phenotype (Du et al., 2004), the present study was undertaken to explore the molecular mechanisms by which H_2S regulates pulmonary smooth muscle cell proliferation and ECM accumulation, and therefore to provide a better understanding of pathogenesis of HPVSR.

Materials and methods

Animal model

The study was approved by the Animal Research Committee of Peking University. The rats were exposed to hypoxia as previously described (Zhang et al., 2003). Twenty-four male Wistar rats (180–220 g) were randomly divided into control group ($n=8$), hypoxia group ($n=8$), and hypoxia with sodium hydrosulfide (hy+NaHS) group ($n=8$). The rats in hypoxia group and hy+NaHS group were exposed to normobaric hypoxia (10% O_2) in a transparent plastic hypoxia chamber for 3 weeks and 6 h every day. Rats in control group were housed in identical cages adjacent to the hypoxic chamber where rats breathed room air. Hypoxia was generated by infusing N_2 into the chamber. The degree of hypoxia was maintained by the balance between nitrogen infusing and the inward leak of air through holes in the chamber. For rats in hy+NaHS group, NaHS dissolved in physiological saline at a dosage of 14 μmol/kg body weight was injected intraperitoneally before hypoxia everyday. The same volume of physiological saline was injected for rats in the other two groups. NaHS solution was freshly prepared by mixing the stock solution of sodium sulfide and hydrochloric acid.

Measurement of pulmonary artery pressure

Three weeks after hypoxic exposure, the rats were anesthetized with urethane (1 g/kg body weight) intraperitoneally. A silicone catheter (outer diameter of 0.9 mm) was introduced into the right jugular vein via a venotomy and passed across the tricuspid valve and right ventricle into pulmonary artery. Another end of the catheter was linked with a Multi-Lead Physiological Monitor (RM-6000, Japan) through P50 pressure transducer. The curve of pulmonary artery pressure was traced and mean pulmonary artery pressure (mPAP) was measured.

Preparation and morphometric analysis of lung tissue

After pulmonary artery pressure was measured, the thoracic cavity was opened. One side of lung lobe was removed out, fixed in 10% (wt/vol) formalin and routinely processed into 5 μm paraffin sections for in situ hybridization and immunohistochemistry.

The small arteries were defined as 15 to 50 μm in external diameter. All small pulmonary arteries per tissue section were assessed at 400× magnification. Each vessel was divided into three types according to the degree of muscularization: muscularized arteries (MA, with two distinct elastic lamina, external and internal), partially muscularized arteries (PMA, with a continuous external elastic lamina and a discontinuous internal elastic lamina), and nonmuscularied arteries (NMA, with only one single elastic lamina). The percentage of each type was determined.

Measurement of H_2S concentration in the plasma

In a test tube containing 0.5 ml of 1% zinc acetate and 2.5 ml of distilled water, 0.1 ml of plasma was added. Then 0.5 ml of 20 mM N, *N*-dimethyl-*p*-phenylenediamine dihydrochloride in 7.2 M HCl and 0.4 ml of 30 mM $FeCl_3$ in 1.2 M HCl were also added to the test tube for 20 min of incubation at room temperature. The protein in the plasma was removed by adding 1 ml of 10% trichloroacetic acid to the solution and centrifuged. The optical absorbance of the resulting solution at 670 nm was measured with a spectrometer (Shimadzu UV 2100, Japan). The H_2S concentration in the solution was calculated against the calibration curve of the standard H_2S solution.

Immunohistochemical analysis

After dewaxed by dimethylbenzene, the sections of lung tissues were put into distilled water and the sections were processed by 3% H_2O_2 for 20 min. The slides were washed by phosphate-buffered saline (PBS) for three times, each for 5 min. Then the antigens were exposed for 15 min. Again the slides were rinsed for three times in PBS, each for 5 min. Then the samples were blocked for 10 min with goat serum working fluid. Polyclonal antibody to collagen I